OTS: 60-11,739

JPRS: 2799

14 June 1960

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Distributed by:

OFFICE OF TECHNICAL SERVICES
U. S. DEPARTMENT OF COMMERCE
WASHINGTON 25, D. C.

Price: $0.50

U. S. JOINT PUBLICATIONS RESEARCH SERVICE
205 EAST 42nd STREET, SUITE 300
NEW YORK 17, N. Y.

JPRS: 2799

CSO: 3837-N

TISSUE RESPIRATION AND GLYCOLYSIS IN THE DEVELOPMENT OF ACUTE RADIATION SICKNESS

/This is a translation of an article written by D. A. Golubentsev and G. V. Sazykin in Voprosy Meditsinskoy Khimii (Problems of Medical Chemistry), Vol. 6, No. 1, Moscow, 1960, pages 49-52./

The condition of tissue respiration and glycolysis in the development of acute radiation sickness has been insufficiently studied, and the factual data and opinions of various investigators are frequently contradictory. One cannot find in the available literature any data on simultaneous investigations of those biochemical processes in many tissues in relation to the development of radiation sickness. I. I. Ivanov and co-authors (1) Piri (2) and others underline the deficiency in our knowledge of those problems.

The purpose of the present work consists in the systematic study of the intensity of tissue respiration and glycolysis in the course of the development of acute radiation sickness of a severe degree.

Materials and Methods of the Investigation

Nonpedigree rabbits weighing from two to four kilograms and white rats weighing from 160-250 g were the object of the investigation. The animals were subject to general X-irradiation under the following conditions: the apparatus -- "Stabilivol't," the tension -- 160 kv, the intensity of the current -- 5 ma, the filter -- 2.5 mm of aluminum, the stratum of half dilution 0.28 mm of copper, the power of the dose in the air -- 21-28 r/min., the total doses of irradiation -- 1,000 and 1,500 r for the rabbits and 800 and 1,000 r for the rats. In a separate series of experiments the general gamma irradiation of the rats was carried out from a cobalt source (Co^{60}) with a dose intensity in the air of 12,500 r/hour; the total dosage of irradiation was 12,500, 14,000 and 25,000 r.

The animals irradiated with doses over 10,000 r were

killed during the first minutes and hours following the action of radiation.

Irradiation with doses of 1,000-1,500 r for the rabbits and 800-1,000 r for the rats was followed by the development of acute radiation sickness of which 80-100 percent of the animals perished within the course of 30 days. The period of the full development of the sickness began upon the fifth-sixth day following the irradiation and persisted up to the 25-30th day. It was characterized by general depression, weakening of the protective reflexes, decrease of alimentary excitability, appearance of sero-sanguinolent excretions from the eyes and the nose, loss of weight (up to 30 percent of the original), by leucopenia (up to 200-500 leucocytes in one mkl of blood) and anemia. Typical morphological changes were demonstrated at the autopsy of the killed and dead animals: aplasia of the bone marrow, and of the lymphoid tissue of the spleen and small intestines, bloody impregnation of the lymphatic nodes, hemorrhages in the internal organs and in the subcutaneous tissues.

The endogenic tissue respiration of sections of the cerebral cortex, of the liver, kidneys, spleen, myocardium, of the thinnest part of the diaphragm, of emulsions of the mucosa of the small intestines, and of the bone marrow was measured manometrically by the method of Varburg; the sections or emulsions of the tissues were incubated for a period of one hour at 37° in the buffered phosphate solution of Krebs-Ringer (pH 7.34) in an atmosphere of oxygen. The anaerobic glycolysis in the tissue emulsions or in the sections was evaluated by the accumulation of lactic acid during an incubation period of two hours in an atmosphere of nitrogen at 37° in the buffer phosphate solution of Krebs-Ringer containing 200 mg percent of starch and 100 mg percent of glucose. The quantity of the lactic acid was determined by the method of Frideman, Kotonio and Sheffer.

The results of the measurements of tissue respiration were expressed in microliters of oxygen taken up during one hour / one mg of raw weight of the tissues, and of the tissue glycolysis -- in mg of lactic acid produced in 100 g of tissue (crude weight) for a period of one hour. Our experiments on a large number of rats, rabbits and mice demonstrated that the content of dry residue in all the investigated tissues did not change substantially in the course of the development of acute radiation sickness.

The numerical data were submitted to statistical processing. The mean arithmetical, and mean quadratic deviation, the mean error (m), and the square root of the sum of square of mean errors (mdiff) were calculated. The changes were accepted as being accurate when the difference of compared arithmetical means was three times greater than

the square root of the sum of squares of the mean errors.

The Result of the Investigations

The level of the endogenic respiration of the spleen, the bone marrow, the mucosa of the small intestines and of other investigated tissues of rats and rabbits did not change materially during the first minutes and hours following general irradiation with doses of 800-25,000 r.

At the peak of radiation sickness, the endogenic respiration of the tissues of the brain, kidneys, lungs, myocardium and diaphragm was not altered substantially; that of the tissue of the liver decreased moderately (on the average, by 15 percent); that of the tissue of the bone marrow and of the mucosa of the intestines and of the spleen decreased markedly (in the rabbits on an average by 70, 31, and 28 percent, respectively. The depression of respiratory activity of the last three tissues coincided in time with the development of their destructive changes. The restoration of the cellular structure of those tissues in the surviving animals was accompanied by normalization of tissue respiration (Table 1).

These results were confirmed later by other investigations. Thus, according to the data of Sullivan and Dubois (3), total irradiation of rats with a dose of 400 r caused a decrease of the endogenic respiration of the tissues of the spleen and of the thyroid gland as well as of respiration at the expense of the added substrata; they noted a maximal decrease of respiration within 48 hours following irradiation with a gradual restoration to normal values on the 15th day.

It was demonstrated in the present work that the anaerobic glycolytic activity of the tissues of the liver, small intestines and of the diaphragm is not altered within 15 minutes and three hours following general irradiation with a dose of 14,000 r and within three, 24 and 48 hours with a dose of 750 r; that of the spleen tissue even increases somewhat (on the average by 15 percent). These data are in accord with the results of the works of Lelievre (4), who investigated the intensity of anaerobic glycolysis immediately after general irradiation of mice with a dose of 60,000 r, and who also failed to demonstrate any changes.

The anaerobic glycolytic activity of emulsions of the spleen of rats decreased on the average by 22 percent and that of emulsions of muscles of the thigh by an average of 27 percent during the peak period of acute radiation sickness of severe degree (sixth-12th day following irradiation with doses of 750 and 1,000 r). (Table 2).

Table 1

The Intensity of Endogenic Respiration of Tissues of Rabbits and Rats in the Course of the Development of Acute Radiation Sickness of Severe Degree

Species of Animal	Dose of irradiation	Time after irradiation (days)	Stage of the Illness	Organ	Number of Animals	Intensity of respiration: Mean $\pm$ m
Rat	no irradiation	--	--	spleen	18	0.98 ± 0.06
	1,000	4-11	acme	"	17	0.62 ± 0.07
	800	29-51	resolution	"	5	0.86 ± 0.10
Rabbit	no irradiation	--	--	"	12	0.85 ± 0.07
	1,500	4-20	Acme	"	15	0.61 ± 0.06
	1,000	23-33	Resolution	"	4	0.82 ± 0.10
Rat	no irradiation	--	--	Intestinal mucosa	14	0.25 ± 0.03
	1,000	4-11	Acme	"	17	0.19 ± 0.02
	800	29-51	Resolution	"	5	0.22 ± 0.04
Rabbit	no irradiation	--	--	"	12	0.67 ± 0.07
	1,500	4-20	Acme	"	16	0.46 ± 0.06
	1,000	23-33	Resolution	"	4	0.62 ± 0.06
Rabbit	no irradiation	--	--	Bone	12	0.20 ± 0.02
	1,500	4-20	Acme	Marrow	16	0.06 ± 0.01
	1,000	23-33	Resolution	"	4	0.21 ± 0.05
Rat	no irradiation	--	--	Liver	19	1.26 ± 0.07
	1,000	4-11	Acme	"	17	1.07 ± 0.06
Rabbit	no irradiation	--	--	"	24	1.16 ± 0.05
	1,500			"	17	1.07 ± 0.06

Table 2

The Intensity of Anaerobic Glycolysis in Tissue Emulsions of Rats During the Peak Period of Acute Radiation Sickness Grade III, Produced by General Irradiation with a Dose of 1,000 r.

Organ	Experimental Conditions	No. of Rats	Constant of Lactic Acid (mg%)	
			Prior to Incubation Mean ± m	After Incubation Mean ± m
Spleen	No irradiation.....	12	88±6	412±12
	Six-11 days after irradiation.....	15	81±7	318±22
	M_1-M_2	--	-7	-94
	$3m_{diff}$.....	--	21	90
Skeletal Muscle	No irradiation.....	11	212±12	580±28
	Six-11 days after irradiation.....	14	211±8	426±16
	M_1-M_2.....	--	--	-153
	$3m_{diff}$.....	--	--	96

The decrease of the glycolytic activity of the spleen tissue is apparently associated with changes in its cellular composition due to the destruction of lymphoid cells which are characterized by a high metabolic activity. The decrease of the intensity of glycolysis in the skeletal muscles may result from a decrease in the concentration of some easily diffnsible coenzymes (codehydrase I) and enzymes (aldolase).

Analysis of Results

The results of the present work demonstrated that general irradiation of rats and rabbits -- even with doses ten times above minimal, absolutely lethal doses -- fails to lower the intensity of anaerobic glycolysis and of endogenic tissue respiration during the first hours in all the investigated tissues. It follows that no inactivation of the enzyme system catalysing these biochemical processes

takes place in those tissues as a result of the direct action of the radiation penetrating into the organism. Since many enzymes of tissue glycolysis and respiration belong to the sulfhydryl group of enzymes, the presented experimental data -- jointly with some data from the literature (1, 5) -- demonstrates the unsoundness of the theory of Barron (6), which assigns a primary significance to the irreversible inactivation of the sulfhydryl enzymes in the mechanism of the development of acute radiation injuries: these data also prove that it is not possible to apply to the living organism any data obtained in experiment with diluted solutions of enzymes. We were unable to confirm the results of the work of Khikman and Eshvell, who found a depression of anaerobic glycolysis of 80-90% within the first hours following general irradiation with a dose of 650 r in homogenates of the spleen of mice.

The results of the present work also conflict with the experimental data of Barron, Wolkowitz and Muntz (6) concerning significant, depression of tissue respiration during the first hours following general irradiation with doses approaching the minimal, absolute lethal dosage. An analysis of the factural data of these authors indicates, however, that there was no regular relationship between the degree of depression of the respiration and the dose of irradiation; in some cases, the changes in respiration following irradiation with a dose of 400 r were of the same degree as those following a dose of 800 and 1,000 r; the greatest changes were demonstrated in the kidney tissue, while in the liver tissue respiration remained unchanged. Our results are in agreement with the results of the investigations of N. M. Sisakyan (7), who demonstrated a high radio-stability of various glycolytic enzymes.

Lelievre demonstrated a high radio-stability (4) of the glycolytic enzyme systems of the tissues of mammals; that of microorganisms was demonstrated by M. N. Meysel' and his collaborators (8).

Conclusions

1. General irradiation of animals with doses up to 25,000 r does not depress tissue respiration and glycolysis during the first minutes and hours.
2. The respiratory activity is sharply depressed during the peak period of acute radiation sickness, Grade III in the tissue of the bone marrow; it is significantly decreased in the tissues of the spleen and of the mucosa of the small intestines; it is moderately decreased in the tissue of the liver; it is essentially unchanged in the other investi-

gated organs (cerebral cortex, kidney, myocardium, lungs and diaphragm).
3. The glycolytic activity significantly decreases in the tissues of the spleen and of the skeletal muscles during the peak period of acute radiation sickness, Grade III.
4. The intensity of respiration in the tissues of the spleen, intestinal mucosa, and bone marrow is restored to normal during the period of clinical recovery.

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Submitted
3 October 1958

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